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A Serial-Dilution Apparatus for Continuous Delivery of Various Concentrations of Materials in Water

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A SERIAL-DILUTION APPARATUS FOR CONTINUOUS DELIVERY OF VARIOUS CONCENTRATIONS OF MATERIALS IN WATER

Donald I. Mount

Aquatic Biology Section
Basic and Applied Sciences Branch
Robert A. Taft Sanitary Engineering Center

and

Richard E. Warner

Engineering Science, Inc.
Oakland, California

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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ABSTRACT

This paper describes a serial-dilution apparatus designed to deliver continuously a series of different concentrations of a material in water. The materials needed for construction normally would be available in a chemistry laboratory. No electrical power is needed for operation, and the apparatus will remain accurate even if the influent waterflow varies over a wide range. It maintains accuracy of 10 percent or less for periods of time up to 30 days or more with very little servicing or adjustment, and the cost is $50 or less.

A SERIAL-DILUTION APPARATUS FOR CONTINUOUS DELIVERY OF VARIOUS CONCENTRATIONS OF MATERIALS IN WATER

Introduction

The evaluation of adverse effects of toxicants or polluting agents on aquatic animals is receiving increasing attention as pollution of the nation's water becomes more severe. This evaluation requires that the aquatic animals be exposed to an environmental concentration rather than to an injected dose of the agent. It is necessary, therefore, to use tests in which the concentration in water of the material being tested is known and maintained constant. These conditions cannot be met in a static or nonrenewed test because the toxicant may volatilize, precipitate, adsorb onto suspended material, or be absorbed by the test organisms, and thus the amount of toxicant in the water would be constantly changing.

Static tests often require additional aeration to maintain the necessary dissolved oxygen (DO) in the water, and test animals cannot be fed because the water becomes foul. Moreover, static tests cannot be continued for more than a few days because metabolites, such as ammonia, accumulate in the water. Periodic renewal of the test water offsets some of the objections to the static test, but the process is laborious and does not sufficiently maintain controlled conditions.

Continuous-flow tests are the best tool available at present to evaluate toxic effects under controlled conditions. For laboratory studies, they most closely approach actual pollutional situations, and are, therefore, being used more commonly by workers in the field. The Newtown Laboratory of the Robert A. Taft Sanitary Engineering Center has been engaged in continuous flowthrough testing for 3 years, and the problem of maintaining concentration control on 100 or more flowthrough test chambers has been troublesome and has required much costly time of technicians. The cost of metering pumps and valves needed to do the job adequately have increased, and malfunctions have occurred regularly. Nearly two full-time positions have been required to maintain and check the systems each day, including weekends and holidays. The most critical fault of the systems in use--a fault that became impossible to tolerate--consisted in a metering system's delivering toxicant solution when the waterflow slowed or failed. The result was the death of the fish in the test chambers. These problems became acute when long-term studies on fish reproduction were begun; a year's work could be lost in one night in this manner. The apparatus described in this paper is a result of efforts to improve dependability of the dosing process.

In 1962, Mr. Richard Warner of Engineering Science, Inc., Oakland, California, in an annual report on a Public Health Service contract, described a serial-dilution apparatus that he had built to conduct flowthrough tests. Serial dilution has many advantages:

It is more accurate in most cases, fewer controlling valves are needed, only one stock solution is required, and there are fewer small constrictions to become clogged. We have used the principles from Mr. Warner's apparatus to build diluters suitable for our use. We now have 13 of these diluters in operation; they have improved our accuracy of concentration control, at least 40 man-hours per week have been saved, the number of malfunctions has dropped sharply, and space requirements have been reduced. In addition, the toxicant dose is dependent on waterflow, the cost of the metering system has been reduced from $600 to $800 to $50 or less, [1] and the system can handle water with plankton or suspended solids of surprisingly large particles and still not clog.

MATERIALS

Supplies normally available in a chemistry laboratory have been used to construct the apparatus. Crystallizing dishes and sidearm vacuum flasks are useful for making the various cells. Epoxy glue is especially convenient for gluing glass tubes in place and making watertight seals. A polyethylene hose "T" can be used for the valve housing.

GENERAL PRINCIPLE OF OPERATION

The serial-dilution apparatus described here consists of three functional components: the water delivery system, the toxicant feed system, and the serial-dilution system. In essence, water delivery is accomplished by filling the water-metering cells, stopping the waterflow to the cells, emptying the metering cells, and restoring the waterflow. This sequence must be completed twice to produce one cycle of the serial-dilution system; water containing the appropriate toxicant concentration is delivered once per cycle. The sequence of delivering 1 unit volume of water from each metering cell, together with the associated changes in the serial-dilution system, is termed a half cycle.

THE SERIAL-DILUTION SYSTEM

To grasp better the major functional concepts of this apparatus, temporarily disregard the water delivery and toxicant feed systems and consider only the serial-dilution system. Refer to Figures 1, 2, and 3 and cells M-1, T-1, and M-2. For illustration, assume a desired volume of 100 milliliters of dosed water for each concentration per cycle. If a 2:1 dilution ratio is used, twice the desired volume of the first concentration must be made to have a sufficient quantity for making other dilutions. At the first half cycle, 100 milliliters is discharged into chamber M-1 along with an appropriate quantity of toxicant, Figure 3A. Chamber M-1 will then be one-half full, and during the next half cycle, an additional 100 milliliters of water is added along with toxicant, giving a total volume of 200 milliliters, a volume that fills cell M-1 and causes the siphon to start. T-1 is adjusted so that the transfer volume (volume of water contained between the levels of the standpipe and the end of the

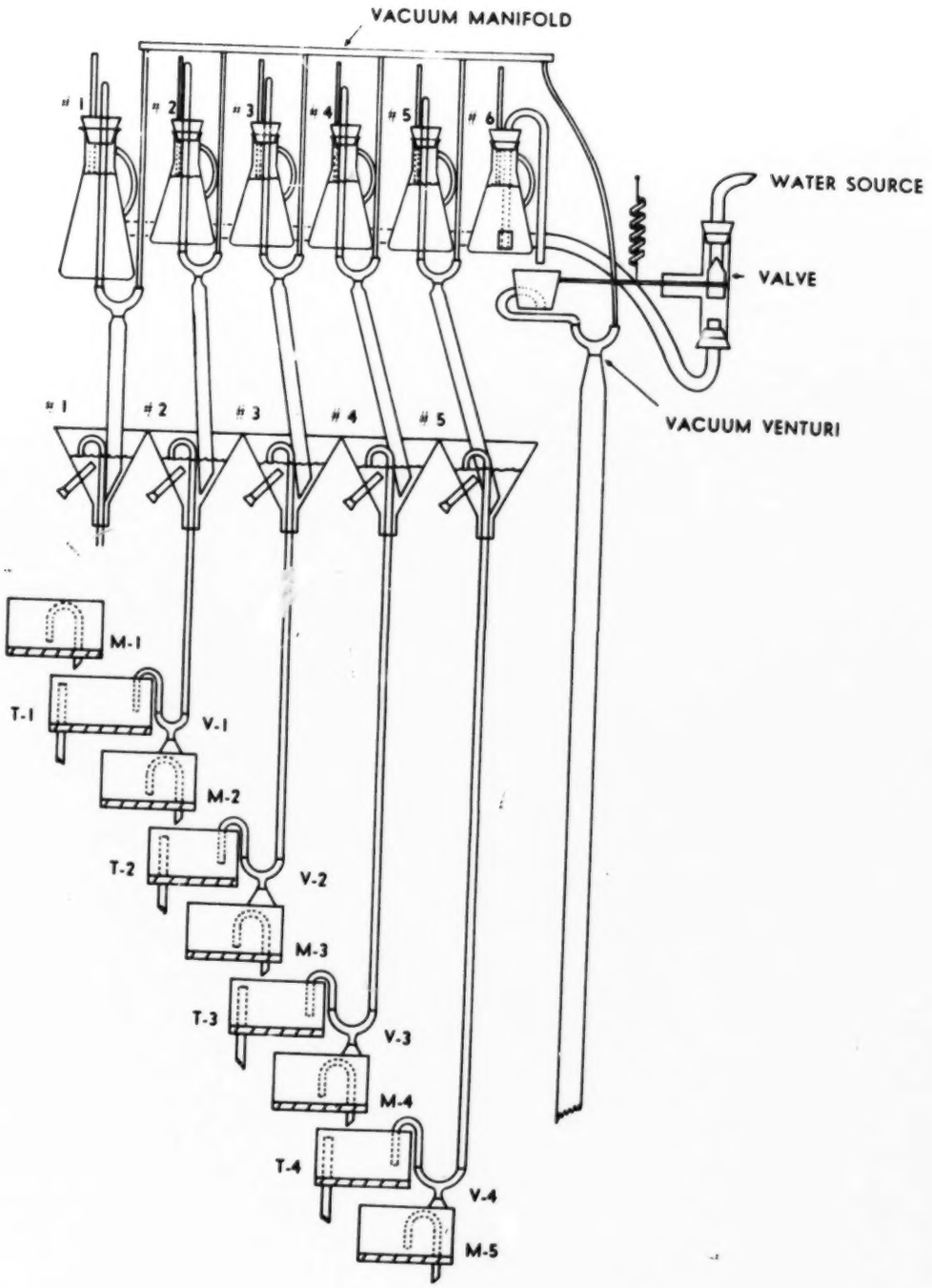

Figure 1. Diagram of the serial-dilution apparatus. Flasks 1 to 6 at the top are metering cells, M-1 to M-5 are mixing cells, T-1 to T-4 are transfer cells, and V-1 to V-4 are venturi tubes. The toxicant-metering system is not shown

venturi siphon tube, Figure 2) is 100 milliliters. As the siphon of M-1 discharges water into T-1, 100 milliliters remains in T-1 and 100 milliliters goes down the standpipe to a test chamber or other location, Figure 3B.

During the next half cycle, 50 milliliters of water is delivered through transfer venturi 1 into cell M-2 (Figure 1). As the 50 milliliters passes through the venturi, the siphon from T-1 to M-2 starts and 100 milliliters of the previous concentration (presently in T-1) is added, making cell M-2 three-fourths full (Figure 3C). Finally, 50 milliliters of water is again added to M-2. Since T-1 contains no

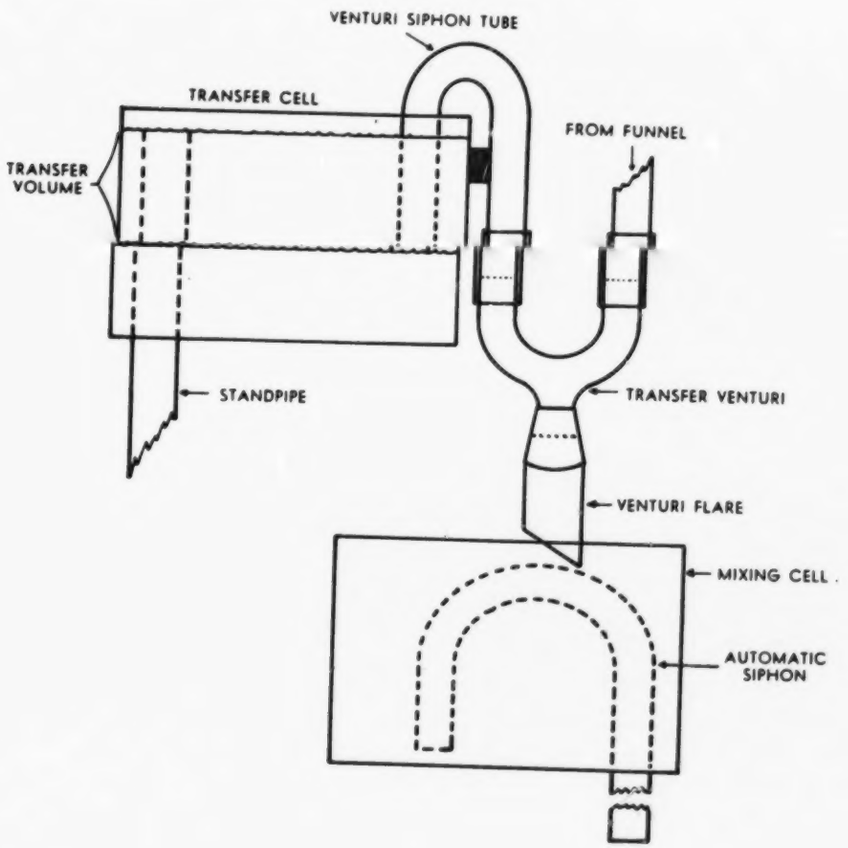

Figure 2. Details of the transfer venturi, transfer cell, and mixing cell.

water that can be siphoned by the venturi siphon tube, none is transferred from T-1 to M-2 during this half cycle. The addition of the second 50-milliliter volume to M-2 fills it, and the siphon starts and empties 200 milliliters of solution into T-2, Figure 3D. This 200-milliliter volume is one-half as concentrated as the first 200-milliliter volume in M-1. During the two half cycles when water was being added to M-2, 100-milliliter volumes were being added to M-1

so that when M-2 is full, M-1 is full again, and both empty simultaneously and fill T-1 and T-2. The same sequence is continued for as many concentrations as are desired. Two-hundred milliliters of the last concentration will be discharged from the "M" cell because no water is transferred downward to make additional concentrations.

Water is discharged on half cycles rather than once each cycle to simplify timing problems. One can see from Figure 3 that all of the 50 milliliters entering the venturi on T-1 must clear the venturi before the 200 milliliters from M-1 fills T-1 again. This is easily accomplished by half-cycle operation, but if water were delivered

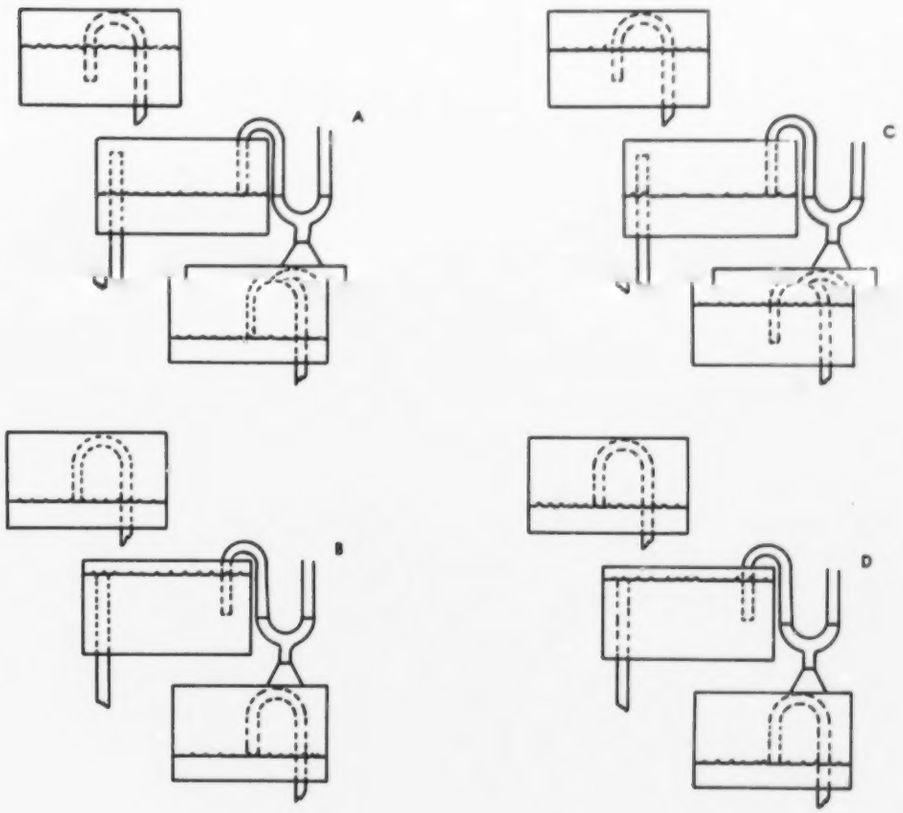

Figure 3. General diagram illustrating the operation of the serial-dilution system.

only once per cycle, 100 milliliters of water from the metering cell and 100 milliliters from T-1 would have to arrive in M-2 before any of the 200 milliliters from M-1 reached T-1. If this did not happen and the venturi siphon on T-1 was still discharging water to M-2 when M-1 began emptying into T-1, all of the water emptied from M-1 to T-1 would subsequently be transferred to M-2 and then on to T-2, and so forth. Water delivery each half cycle makes possible a safety factor that prohibits the action referred to above and that is called "double siphoning."

In addition, by using half-cycle delivery, the diluter need not remain "in phase." "In phase" refers to sequences in which all "T" cells are full and all "M" cells are empty. After the next "in phase" half cycle, all "M" cells are half full and all "T"-cell transfer volumes are empty. By using half-cycle delivery, cells T-1 and T-3 may be full while the transfer volumes of T-2 and T-4 are empty. Consequently M-1, M-3, and M-5 would be empty and M-2 and M-4 would be half full when the "T" cells are as described above. The condition that must be met is that when a "T" cell is full, the "M" cell immediately above it must be empty, and when the transfer volume of a given "T" cell is empty, the "M" cell immediately above must be half full. Each diluter usually operates in a particular pattern called the phase sequence pattern. The latter one described above is called the alternating sequence pattern and is the more common because timing is less critical.

In practice, the total volume need not be used on any cell. One can shorten the siphon tube in the "M" cells, for instance, and use only half of their volume. In this instance the "M" cells would never empty completely. If a dilution of 1:3, or any other ratio, is desired, the volume transferred from the "T" chambers to the "M" chambers is changed accordingly to give the desired ratio. An example would be a transfer volume of 75 milliliters and a half-cycle water delivery of 112.5 milliliters to M-1 and 75 milliliters to the others.

Figure 2 shows the details of the "T" cell and the "T"-cell venturi. A very efficient venturi can be made from either "T"- or "U"-shaped glass connecting tubes if they are cut and modified as shown. One important precaution is that the venturi flare must have the same or slightly larger bore at its upper end as the bore of the venturi "U" tube. If the upper end of the flare is smaller, a back pressure will develop on the venturi siphon tube and the siphon will not start.

Another suggestion for better venturi operation is the use of a tube bore for the funnel siphon and funnel siphon tube (Figure 4) that is slightly smaller than the bore of the "U" tube venturi. The funnel siphon tube must fill with water without air for proper venturi operation. The primary purpose of the funnel siphon is to keep air out of the funnel siphon tube. Many of our diluters operate without these funnel siphons because the funnel siphon tube is small enough and the metering cells empty fast enough to prevent air from entering the funnel siphon tube.

Finally, the venturi flare must not be excessively large or else the water and air mixture (the air enters from the venturi siphon tube until the water from the "T" cell arrives) passing through the flare will not completely fill it and there will be no venturi. An improper taper on the flare or a nonwettable film such as grease can cause the same type of trouble.

WATER DELIVERY SYSTEM

Other types of water delivery systems were tried in conjunction with the serial-dilution system, but these would not deliver water with

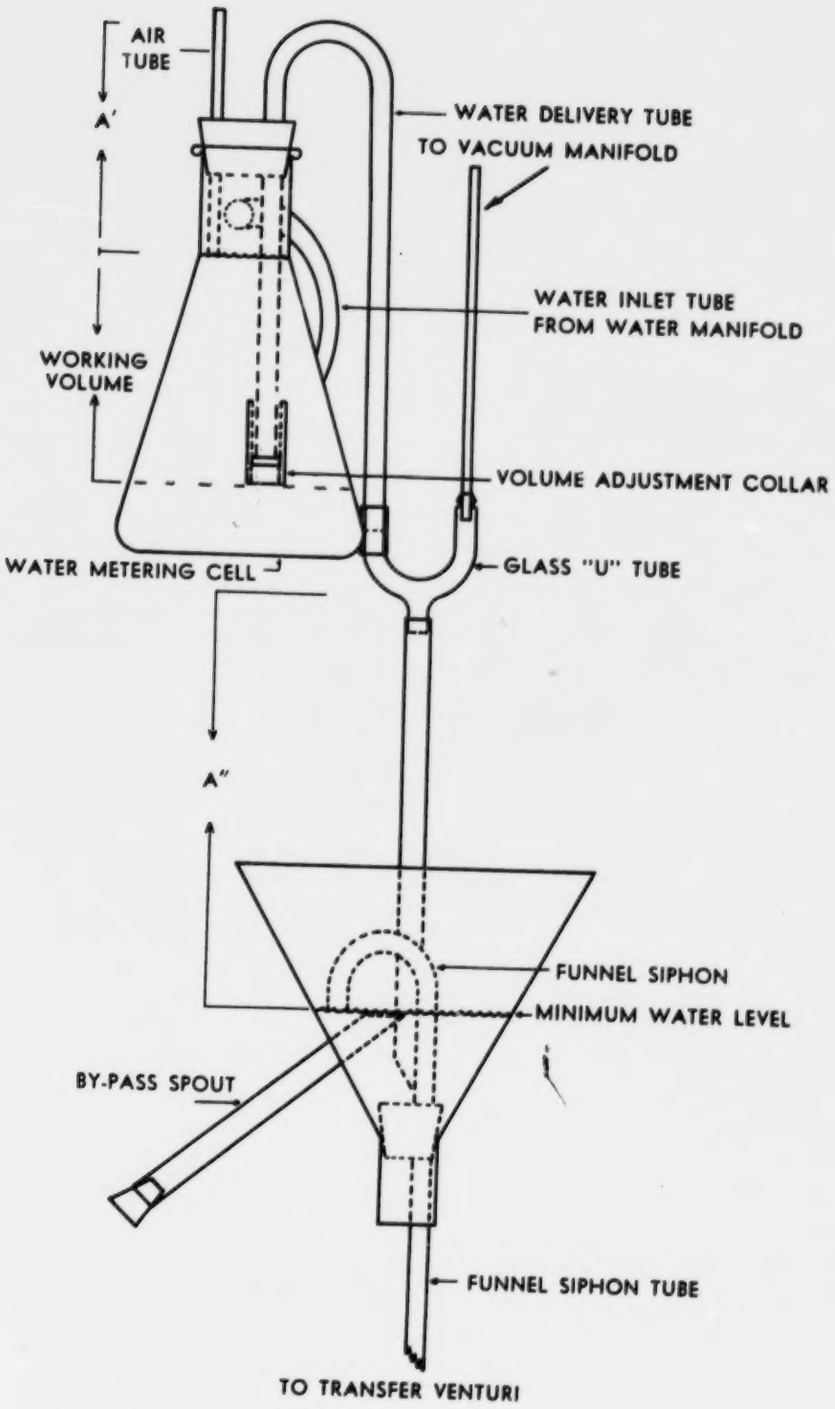

Figure 4. Detail of a water delivery unit.

an accuracy comparable to that achieved by the serial-dilution system. This system has the advantage that one can visually determine whether or not the proper volume of water is being delivered, with an error of 10 percent or less. More important, the volume delivered each half cycle is not affected by the rate at which water is fed to the system and there are no small water passages that can clog with solid material.

As shown in Figures 1 and 4, the water delivery system is composed of a series of units, each consisting of a metering cell with a water inlet, air tube and water delivery tube, a venturi, and a funnel. There is a common vacuum manifold, vacuum venturi, and water shut-off valve.

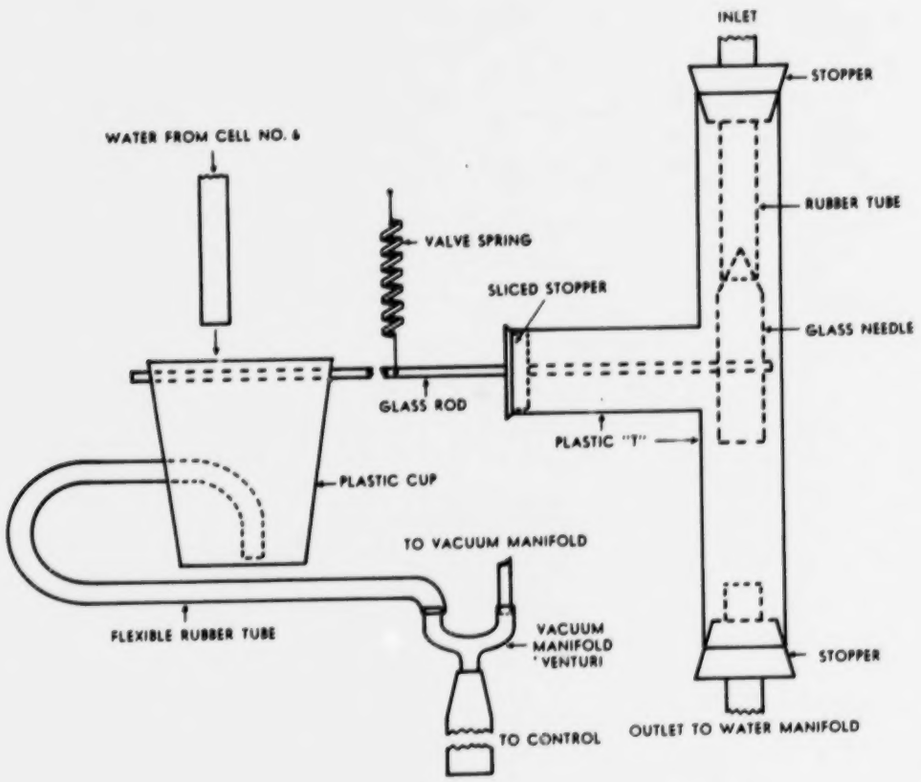

Figure 5. Detail of the main water valve and vacuum venturi.

Frequent reference to Figures 1, 4, and 5 will assist in understanding the operation. A water manifold delivers water to each of the metering cells, which fill until the water level is at the end of the air tube. Cell Number 6 fills last because the inlet to it is higher than that of the others, and it empties first because its outlet is lowest. As water from the last cell fills the bucket on the needle valve arm, the valve is closed by the additional weight. When the bucket is filled

to the level of the siphon tube, the water travels through the vacuum venturi, producing a partial vacuum in the vacuum manifold, and is then discharged to the control chamber. The reduced pressure in the vacuum manifold causes water to rise from both ends of each water delivery tube (from the funnel and from the metering cell). If distance A' is less than distance A" (Figure 4), the water will rise over the arch of the tubes and activate the siphons, which will empty the metering cells down to the end of the water delivery tube. By the time the siphons break, the bucket on the needle valve arm will be nearly empty, the valve will open, and the metering cells will fill again.

The sequence in which the metering cell siphons start is determined by the realtive distance of A' of each cell. Figure 1 illustrates the most desirable order, namely, Numbers 1 and 5 start at nearly the same time and then Numbers 4, 3, and 2 begin in that order. Number 1 should begin emptying early because if a 2:1 dilution is used the first cell must deliver twice as much water as the other cells.

The "funnel end" of each water delivery tube must remain under water at all times so that a partial vacuum can be created in the vacuum manifold. A bypass spout on the funnel is used to measure the water delivered by the cell during operation. The funnel siphon and bypass spout are positioned so that when the bypass is open enough water remains in the funnel to cover the end of the water delivery tube and so that all the water delivered goes through the bypass and none goes over the funnel siphon to the transfer venturi.

Major volume adjustments of the metering cells are accomplished by moving the volume adjustment collar (Figure 4), and minor ones are made by moving the air tube. Caution must be exercised, however, since moving the air tube changes distance A', which could change the sequence of delivery. If changed enough, the distance may exceed A" and then the siphon will not start.

Care should be taken in constructing the vacuum venturi to see that proper tube sizes and tapers are used to produce sufficient vacuum. If it is properly made, more than enough vacuum can be produced with ease.

TOXICANT-METERING SYSTEM

The toxicant-metering system is not necessarily an integral part of the apparatus. We have used several other types such as metering pumps and controlled dripping rates but all have been inferior to the one shown in Figure 6. It has the important advantage that it will not deliver toxicant unless waterflow is near normal.

The system as shown in Figure 6 is nearly self-explanatory. The solution level in the funnel is kept constant by some means such as a Mariotte bottle or float valve. If the toxicant is a suspension rather than a solution, settling may occur. A small peristaltic pump may be used to pump the suspension to the funnel with a bypass, overflow line back to the bottle. In this way the level in the funnel is maintained and the suspension is mixed and replaced, which eliminates settling.

For use on the diluter apparatus, the plastic bucket of the metering system is placed beneath the first funnel of the diluter (Figure 1) and when water flows through the funnel siphon tube the bucket is filled with water. This weight causes the bucket arm to drop and the opposite arm rises upward taking with it a predetermined volume of toxicant, which runs into M-1 through the tube arm.

The components are easily assembled and the tube is simply made by heating an appropriately sized piece of glass tubing in a narrow area, stretching it until the bore is sufficiently reduced, cooling, and then bending the two angles. The bore is constricted so that minor fluctuation in the solution level in the funnel will not significantly change the volume delivered.

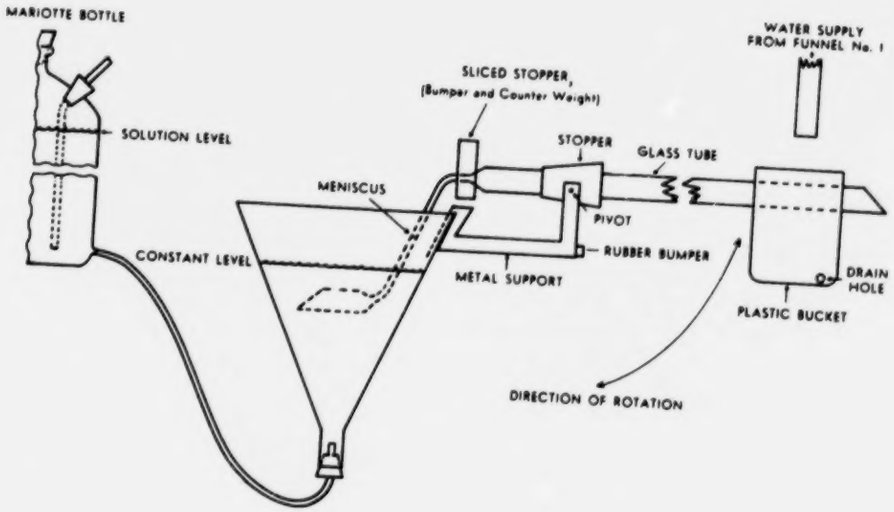

Figure 6. Detail of toxicant-metering system. Bottle is not in proportion to drawing.

The accuracy of this system equals or exceeds the accuracy of systems using metering pumps and constant waterflows because delivery is balanced against a water volume rather than a flow; thus a major source of variation (waterflow) is bypassed. Obviously, for best calculations one should know how many times the metering cell empties and likewise how many times toxicant is added. Since it did not seem advisable to put a counter switch on the metering system because of its small size, a microswitch was placed on the needle valve arm (It moves each time the Number 1 cell empties.), giving an actual count of the number of times chemical is delivered. (Mechanical counters are also very good because no electricity is needed.)

By using a calibrated toxicant bottle, the total volume of toxicant delivered can be read, the number of counts can be obtained from the counter, and the average volume of toxicant delivered each half cycle can, therefore, be calculated. Then, by measuring the volume of water delivered by metering cell 1, the calculated accuracy of the first concentration can be determined.

CALIBRATION

Calibration of the various volumes seems to be a laborious task, but it can be completed in 10 to 15 minutes with experience. After the wetting characteristics of the tubes have stabilized, the calibration needs to be checked only every 2 to 4 weeks to ascertain that the maximum error is 10 percent or less. The diluter should be checked visually every day to insure good operation and detect malfunctions such as that caused by a broken component.

Only two sets of volumes need to be known in order to calculate the error for each concentration, the first excluded. These are the amounts of diluent water being added at each half cycle and the transfer volume (the volume of the next higher concentration being mixed with the diluent water from the water-metering cells).

The volume of diluent water added is checked by opening the bypass on the funnel and catching the volume for one or more half cycles for each metering cell. One must be careful to see that all water delivered goes through the bypass and that none goes over the funnel siphon.

The transfer volume is checked as follows: Assume a desired volume of 100 milliliters. Fill a 250-milliliter graduated cylinder to some volume greater than 120 milliliters. Wait until the "T" cell to be checked is just emptied by the venturi siphon. Pour water into the "T" cell until it is full, then add 20 milliliters or more in addition and catch the water that runs down the standpipe before the next half cycle begins. Return this water to the graduated cylinder. Read the volume now remaining in the cylinder; the difference between the present volume and the original volume represents the transfer volume of the "T" cell being checked. When adding the water to the "T" cell, one should fill it at approximately the same rate as it is filled by the "M" cell siphon above it in order to simulate actual operation.

After all volumes are recorded, the error for each concentration can be calculated. Obviously, the error for the first concentration must be determined by using the volume of water delivered from metering cell 1 and the volume of toxicant delivered by the metering system.

Each diluter will usually operate in a typical phase sequence. Obviously, checking the volumes as described above disrupts this sequence because water is being added or removed. It is best, therefore, to check the transfer volumes first, starting with the lowest transfer cell, and then check the metering cell volumes. In this way each transfer volume is checked when the diluter is in its typical phase sequence. The checks can be made while the diluter

is operating, and the removal or addition of water for checking will cause one or at most two errors. The diluter will correct itself and continue properly, but possibly in a different phase sequence. If a diluter is well timed it will stay in any phase sequence in which it is placed. This pattern offers a useful visual check of proper operations. If the pattern keeps changing every few minutes, the diluter is making errors.

Other visual checks that can be made for proper operation are:

1. Determine whether, when an "M" cell is empty, the "T" cell immediately below it is full. (This is a check for double siphoning. See page 5.
2. Observe the sequence in which water runs down the standpipes of the "T" cells. This should occur on each second half cycle. A malfunction may indicate that a "T" cell venturi is not working.
3. Observe whether the needle valve is turning off the water completely and for a period long enough to allow the metering cells to dump.
4. Observe whether each metering cell is filling and emptying completely each half cycle. Other checks will be learned by experience. These checks may seem tedious, but with experience, a 2-minute daily visual check is sufficient to insure good operation regularly.

TIMING

In performing the calibration, one may find that the system may need to be timed more accurately. This may also be necessary after the wetting characteristics have stabilized. Timing adjustments solve the double-siphoning problem mentioned on page 5. When a diluter is optimally timed, the serial-dilution system can be disrupted for some reason such as for a calibration check or accidentally by dirt, air, and so forth, and the system will make only one error and then return to proper operation. This ability to correct mistakes is highly important because during normal operation things will occur that cause an error and only if the system can correct errors and not repeat them can it be considered satisfactory.

To review: Double siphoning occurs when all the water from a given metering cell has not passed the transfer venturi as the associated "T" cell begins to fill from the next higher "M" cell. The result is that the venturi siphon begins to remove water from its "T" cell when it should not.

There are three timing adjustments: (1) The sequence in which the metering cells empty, (2) the volume of water required to start the funnel siphons, and (3) the volume required to start the "M" cell siphons. If the sequence in which the siphons of the metering cells start is as described earlier, then frequently no other adjustment is needed. If difficulty is experienced in getting the desired sequence or if an additional safety factor is desired, or both, the next best

timing adjustment is the "M" cell volume. The volume of the cell is reduced by shortening the siphon tube or by adding volume displacers such as marbles, in order to make the cell empty sooner, or the tube length is increased, or volume displacers are removed to make it empty later. As a last resort, and this is rarely necessary, one can change the volume of water required to start the funnel siphon in a manner as described above for the "M" cells.

As a general rule, the greatest safety factor for timing should be given to the higher cells. In practice, calibration and timing must be done more or less simultaneously, starting at the top "M" cell and working downward. After the metering cells have been calibrated and one feels that the apparatus is properly timed, the serial diluter should be intentionally fouled by randomly adding water in order to be certain that the diluter can correct errors and resume proper operation.

TROUBLE SHOOTING

Experience in the use of the diluter has shown how to correct minor malfunctions with ease by using "tricks of the trade." Some of these do not fit well into the text and are presented here for the reader's convenience.

1. Sympton: One or more metering cells are not filling or emptying completely.
   Trouble: Dirt in the water or air tubes.

2. Sympton: Last metering cell (No. 6 on Figure 1) fills and empties but the others remain full.
   Trouble: One of the funnels has insufficient water in it and the water delivery tube is getting air, or there is dirt or a break in the vacuum venturi or manifold.

3. Sympton: One or more metering cells are delivering water continuously and other cells are not filling.
   Trouble: Needle valve is not closing tightly, or is not remaining closed long enough for all cells to empty, or a particle of dirt caused the valve to malfunction. Correction may involve adjusting valve seating, providing more water to increase closed period of valve, or improving efficiency of vacuum venturi.

4. Sympton: Water rises over water delivery tubes before valve bucket fills and closes valve.
   Trouble: There is too much water pressure for existing adjustments or there is dirt in water tube leading to metering cell 6. Correction may involve increasing the distances A' (Figure 4), lowering the level of cell 6, increasing the bore of the water manifold and inlet tubes to metering cells, or lowering curve of water delivery tube of cell 6.

5. Sympton: Cell 6 fills before the other cells are full.
   Trouble: Cell 6 needs to be raised slightly.

6. Sympton: Volumes delivered by one or more metering cells vary.
   Trouble: Valve is not closing properly.

7. Sympton: Diluter is double siphoning.
   Trouble: Improper timing.

8. Sympton: Diluter does not hold a phase sequence pattern.
   Trouble: Timing is improper, one venturi is not operating, one metering cell does not always empty, or one of the water volumes (either from metering cell or a transfer volume) is grossly wrong.

9. Sympton: A transfer venturi does not work every time.
   Trouble: There is air in funnel siphon tube, dirt in water line, venturi "U"tube is cracked, flare is not completely filling with air-water mixture, or end of flare is under water of next lower "M" cell part of the time.

10. Sympton: Transfer volumes vary from day to day.
    Trouble: Venturi siphon tubes are not held securely.

PERFORMANCE

One may expect no more than 10 percent calculated error (1 to 3 percent for any one concentration) from a diluter if it is calibrated once every 2 to 4 weeks with daily observations as described previously. One advantage is that the errors tend to be both positive and negative so that they cancel each other. Table 1 illustrates the usual accuracy obtained from a diluter with no special efforts or precautions. These data were recorded in a record book during routine testing and do not represent unusual accuracy nor were they recorded for demonstration purposes.

Table 1. THE CALCULATED AND POLAROGRAPHICALLY MEASURED CONCENTRATIONS OF ZINC IN mg/liter FROM A SERIES OF THREE 4-day TESTS. DAILY SAMPLES WERE COMPOSITED FOR 4 days AND THEN THE ZINC ANALYSES WERE MADE. WATER FLOW WAS 100/ml/min/concentration. THERE WERE 10 FISH IN EACH 10-liter TEST CHAMBER. ALL SOURCES OF ERROR INCLUDING DILUTER ERROR, ERROR IN THE TOXICANT-METERING APPARATUS, WATERFLOW VARIATION, REMOVAL BY THE TEST ORGANISM, AND ANALYTICAL ERROR ARE REFLECTED IN THESE MEASUREMENTS.

Test 1		Test 2		Test 3	
Calculated	Measured	Calculated	Measured	Calculated	Measured
33.5	34	33.4	34.9	41.3	41.0
17.0	18.7	17.2	18.5	20.4	20.5
8.4	10.5	8.4	8.5	11.0	10.9
4.2	5.3	4.3	3.4	5.8	5.5
2.1	2.4	2.1	2.1	2.9	3.0
1.0	1.1	1.1	1.2	1.5	1.4

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The choice of optimum cell size can be made by a convenient rule of thumb as follows: Determine the flow desired for each concentration per minute and use "T" and "M" cells that hold four times that volume. In this way one can obtain a 2:1 dilution ratio by using a 1-minute half-cycle time. The flow can be doubled by decreasing the half-cycle time to one-half minute, about the maximum practical speed, and, of course, the flow can be decreased to any desired volume by simply increasing half-cycle time. One-minute half cycles seem to be optimum.

Figure 7. A serial-dilution apparatus designed to deliver 300 ml /min of five concentrations and a control. Several minor changes are used on this diluter.

Some of our diluters have run for 6 months with no more than one or two adjustments during that period. Of course there were occasions during that period when foreign material or an accident caused a significant error for a short period. Figure 7 illustrates a 5-concentration diluter designed to deliver up to 300 milliliters per minute.

One may logically expect to build and calibrate one of these diluters in a week, but after several are built, this time may be reduced to 3 days or even less. Four professional biologists in the Newtown Laboratory have become proficient with these systems and are thoroughly convinced of their utility. Most recently we have been studying effects of silt in the water, and the diluter appears to be well suited for that work as well.

The experiences of our people with the diluter have been very similar; after the diluter is built, a short period is needed for final adjustments and for solving minor problems. Then, as the operation becomes smooth, satisfaction is felt because technical problems are minimal and the biological investigation can proceed.

REFERENCE

1. Lemke, A.E., and D.I. Mount, Some effects of alkyl benzene sulfonate on the Bluegill, Lepomis macrochirus. Trans. Am. Fish. Soc., 92(4): 372-378, Oct. 1963.

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